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Investigation of Single and Joint Fumigant Insecticidal Action of Citruspeel Oil Components

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Abstract: Following gas chromatographic analysis of citruspeel oils, 30 components of these oils were tested against *Callosobruchus maculatus* (F.). Several compounds (22), including the major component of all citruspeel oils—(+)-limonene—were found to be bioactive, having a strong vapour insecticidal activity. These components and some of their isomers were shown to have varying levels of activity (α-terpineol, 24-h LC_{50} 4·05 μl litre^{-1} to *n*-tridecane, LC_{50} 13·3 μl litre^{-1}), but no single component could account for the activity measured for a typical citruspeel oil (lime) on a proportional basis. Joint action studies established that in artificial mixtures several pure components of citruspeel oil potentiated their individual fumigant action in a manner consistent with an additive model against *C. maculatus*.

Key words: joint fumigant action, citruspeel oil components.

1 INTRODUCTION

Extracts from the epicarp of citrus fruit peel which contain sacs yield essential oils (plant oils with essences—aroma). Citrus essential oils in general contain mainly terpene hydrocarbons and oxygen-containing compounds, including aldehydes, esters and alcohols. It has been shown[1] that filtered lime oil consisted of the following major fractions: monoterpenes, 76%; sesquiterpenes 3·8%; oxygen-containing compounds 18·1%, and each of these fractions is composed of a wide range of compounds. The individual components (over 100) and their quantitative presence in citrus oils have been compiled.[2] Essential oils are used in the flavouring of all kinds of beverages, confectionaries, baked goods and in the perfumery, antiseptic and cosmetic industries.[3,4] While citruspeel oils produce valuable revenue for some tropical countries such as Brazil, Mexico, Gambia and South Africa, in others, like Nigeria, the peels are lost as waste.[5] Therefore, the insecticidal properties of these citruspeel oils may provide more opportunities for economic exploitation of such plant products, particularly over the tropics. The vapour toxicity of citruspeel oils should reduce the amount of bulky peel materials[5] or oils required for the control of storage pests.

The toxicity of citrus oils to insects has been traced to some of their component chemicals. For example limonene and some lesser components showed contact toxicity to *Callosobruchus maculatus* (F.) and houseflies, *Musca domestica* L., in dry film filter paper tests,[6] while citrus oils applied by dipping were more toxic than limonene to eggs of the Caribbean fruit fly *Anastrepha suspensa* (Loew).[7] Most of these reports have involved simple tests to demonstrate the bioactivity of oil components, and none has attempted to explain the activity (vapour or contact) of oils on the basis of individual components or of several components acting together.

Mixtures of several closely related bioactive compounds are often produced by plants and synergism between them may occur.[8] Following gas chromatographic analysis of some citruspeel oils, this work investigated the vapour activity of citruspeel oil components alone and as simple mixtures against stored product pests in order to explain the fumigant action of the whole citruspeel oils.

2 EXPERIMENTAL METHODS

2.1 Test compounds

Thirty components of citruspeel oils (based on results of gas chromatographic analysis) were purchased from Sigma, UK.

Industrially extracted citruspeel oils were obtained from Zimmerman Hobbs Ltd, UK.

2.2 Insect cultures

Stock cultures of strains of *C. maculatus*, *Sitophilus zeamais* Motsch. and *Dermestes maculatus* Deg. which had been cultured in the absence of insecticides for several years were obtained and re-cultured as described elsewhere.[9] All insect cultures were kept at 28°C and 70% RH under constant red light. All bioassays were conducted under these same conditions of temperature, humidity and light.

2.3 Gas chromatography of citruspeel oils

Gas chromatographic (GC) analysis was carried out at the Zimmerman Company Laboratory, Milton Keynes, UK. A Perkin Elmer Model 8329 was used with a fused silica capillary column (0·22 mm ID.; 0·33 mm OD.; x20 m; SGE Code 12 Q C_2) with bonded phase OV101. The carrier gas was helium at 8·3 psi. The capillary inlet and detector temperature was 25°C. Oven temperature was held at 55°C for 5 min then raised to 220°C at 3°C per min.

2.4 Fumigant activity of citruspeel oil components

Fumigation bioassays were carried out in air-tight kilner jars (500 ml), applying oils to Whatman filter paper (3 cm diam.). The glass chambers were sealed with a screw ring holding a glass lid onto a rubber washer covered with aluminium foil to prevent reaction with test compounds.

2.4.1 Fumigant action of citrus oil components against stored product pests

For *C. maculatus* adults (one- to two-day-old, mixed sexes), test insects (15 per chamber) were fumigated with each of 30 components selected for initial screening at 10 and 20 μl litre^{-1}. Mortality was assessed immediately after 24-h fumigation periods.

In a second experiment, compounds that showed insecticidal activity were re-tested, each of them at several concentrations from 2 to 16 μl litre^{-1}. At least 30 insects were used per treatment. Solid components were tested at dosages from 4 to 10 mg litre^{-1}. Mortality was assessed soon after 24 h of fumigation.

2.4.2 Relative fumigant action of (+)-limonene (major constituent of all citrus oils)

Three stored product pest species were tested against (+)-limonene as follows: Test insects (15 adults per chamber) were fumigated for 24 h at several concentrations (*C. maculatus* 6–10 μl litre^{-1}; *S. zeamais* 12–24 μl litre^{-1}; *D. maculatus* 10–24 μl litre^{-1}). Each treatment was replicated twice and mortality was assessed immediately after 24 h of fumigation.

2.5 Joint fumigant action of citrus oil components

Artificial limepeel oil mixture: This mixture was made using four insecticidal components, (+)-limonene + γ-terpinene + terpinolene + α-terpineol (55·0 + 10·6 + 5·3 + 5·8 by mass) in the proportions in which they occurred in the test batch of limepeel oil (Section 2.3., GC analysis). *n*-Decyl alcohol, a non-insecticidal component was included as a filler to make up the proportion of the natural oil (23·3% by mass) occupied by minor components not included in the present tests.

Test insects (15 one- to two-day-old *C. maculatus* adults per chamber) were fumigated with the prepared mixture at rates from 6 to 10 μl litre^{-1}. Each treatment was replicated twice and mortality was assessed after 24 h fumigation. For comparative purposes, other batches of insects from the same culture were fumigated simultaneously (in separate chambers) with natural limepeel oil or binary mixtures (consisting of each of the toxic components in the artificial limepeel oil mixture with *n*-decyl alcohol, at rates equivalent to the artificial mixtures.

2.6 Statistics

(a) All dose-response (mortality) data were analysed using a computer package for probit analysis which

TABLE 1
Fumigant activity of Liquid Citrus Oil Components against *Callosobruchus maculatus* Adults
24-h Mortality (95% CL)

Treatment	LC_{50} (μl litre^{-1})	LC_{95} (μl litre^{-1})	Slope (±SE)	χ^2	DF	Proportion in oil (%)[a]	Expected toxicity ratio[a,b]
Limepeel oil[c]	7·77 (7·11–8·50)	12·94 (10·66–15·68)	7·46 (±1·88)	13·57	4	—	1
Liquid components[d]							
(+)-limonene	8·28 (7·93–8·67)	11·15 (10·30–12·73)	12·74 (±1·85)	3·07	1	54[e]	1·9 (0·9)
Citronellal	5·52 (5·00–5·90)	8·86 (8·00–10·38)	7·99 (±1·14)	7·39	3	0·09[e]	>1100 (1·4)
n-Decyl alcohol	—[f]	—	—	—	—	<0·1	>1000
n-Tridecane	13·13 (12·45–14·43)	18·29 (16·00–25·20)	11·43 (±2.48)	0·30	1	<0·1	>1000 (0·6)
Nonane	—	—	—	—	—	—	—
n-Undecane	10·44 (9·74–10·85)	13·11 (12·19–16·33)	16·68 (±4·5)	0·09	1	0·03	>3300 (0·7)
α-Pinene	10·21 (9·88–10·57)	12·73 (12·00–13·98)	17·16 (±2·4)	0·19	1	1·7[e]	59 (0·8)
α-Terpinene	8·03 (7·75–8·33)	9·97 (9·30–10·66)	17·61 (±2·7)	6·16	2	1·3[e]	77 (1·0)
β-Myrcene	9·61 (9·33–9·89)	11·44 (10·97–12·20)	21·80 (±2·9)	1·13	3	1·3[e]	77 (0·8)
Citral	4·18 (3·70–4·60)	7·78 (6·76–9·85)	6·11 (±0·9)	1·21	1	<0·1	>1000 (1·7)
6-methyl-5-hepten-2-one	4·52 (4·21–4·82)	6·86(6·23–7·97)	9·07 (±1·2)	1·42	1	<0·1	>1000 (1·7)
γ-Terpinene	5·65 (5·13–6·03)	8·69 (7·91–10·03)	8·79 (±1·5)	0·46	1	10·4[e]	10 (1·4)
Geraniol	13·06 (12·48–13·75)	18·03 (16.47–21·09)	11·73 (±1·7)	0·98	1	0·1[e]	1000 (0·6)
n-Decylaldehyde	—	—	—	—	—	<0·1	>1000
Linalool	5·16 (4·91–5·41)	6·67 (6·28–7·37)	14·81 (±2·1)	0·89	3	0·2[e]	500 (1·5)
Toluene	—[f]	—	—	—	—	—	—
Acetone	—[f]	—	—	—	—	—	—
Undecenal	12·21 (11·32–14·73)	18·88 (15·33–36·95)	8·69 (±2·38)	0·13	1	<0.1	>1000 (0·6)
β-Pinene	8·37 (8·03–8·70)	10·67 (9·99–12·02)	15·56 (±2·53)	3·76	2	2·3[e]	44 (0·9)
Terpinolene	6·36 (5·89–6·86)	10·46 (9·12–11·98)	7·63 (±1·12)	10·24	3	5·2[e]	19 (1·2)
Myrcene	9·40 (9·04–9·73)	11·91 (11·28–13·03)	15·97 (±2·26)	0·69	1	1·3	77 (0·8)
Nonanol	—[f]	—	—	—	—	0·5[e]	200
9-Decenal	7·29 (6·82–7·79)	10·52 (9·11–12·13)	10·38 (±2·1)	6·20	1	0·03	>3300 (1·1)
Methyl anthranilate	5·21 (4·88–5·52)	7·64 (7·01–8·77)	9·92 (±1·4)	2·23	3	0·03[e]	>3300 (1·5)
α-Terpineol	4·05 (3·56–4·48)	8·06 (6·60–12·62)	5·50 (±1·14)	5·67	2	5·7[e]	18 (1·9)

[a] Expected toxicity ratio = (100%/% of component in oil) (toxicity ratio if component alone were toxic in oil).
[b] Observed toxicity factor (limepeel oil as reference) in parentheses.
[c] Limepeel oil—typical citrus oil—as reference.
[d] Data contradict the hypothesis of parallelism.
[e] Obtained by GC. Other values from literature.[2]
[f] A: not active.

TABLE 2
Fumigant Toxicity of Solid Citrus Oil Components against *Callosobruchus maculatus* Adults

Treatment	24-h Mortality[a] at concentration (mg litre^{-1}) 4	6	10	0·0	Proportion in oil (%)
Camphene	0	3	4	0	0·56[b]
Fenchyl alcohol	23	30	30	0	0·81
Caprylic acid	NA[c]			0	<0·1
Thymol	NA			0	<0·1
(−)Borneol	0	9	4	0	0·6

[a] $n = 30$.
[b] Proportion in oil obtained by GC analysis. Others values obtained from literature.[2]
[c] NA = Not active against *C. maculatus*.

TABLE 3
Relative Fumigant Toxicity of (+)-limonene against Test Insects

Treatment	24-h Mortality (95% CL)					
	LC_{50} (μl $litre^{-1}$)	LC_{95} (μl $litre^{-1}$)	Slope ($\pm SE$)[a]	χ^2	DF	TF[b]
C. maculatus						
(+)-limonene	8·28 (7·93–8·67)	11·15 (10·30–12·73)	12·74 (±1·85)	3·07	1	1
S. zeamais						
(+)-limonene	16·22 (15·35–16·94)	22·04 (20·54–24·87)	12·35 (±1·90)	2·73	2	0·51
D. maculatus						
(+)-limonene	15·35 (14·32–16·46)	23·16 (20·58–28·87)	9·21 (±1·56)	3·42	2	0·54

[a] Data do not contradict the hypothesis of parallelism.
[b] Toxicity Factor (reference (+)-limonene—*C. maculatus*).

included tests for parallelism and relative potency based on accepted procedures.[10]

(b) The joint fumigant action of citruspeel oil components: A general eqn (1) (after Hewlett and Plackett)[11] was used to calculate the expected dose (Z) of a mixture of insecticidal components which will produce some specified response in a target population.

$$\frac{C(1)}{Z(1)} + \frac{C(2)}{Z(2)}\frac{C(3)}{Z(3)} \cdots \frac{C(n)}{Z(n)} = \frac{1}{z} \qquad (1)$$

Where $C(1)$ is the proportion of component 1 in the mixture and $Z(1)$ is the dose of component 1 required to produce the same effect in the target population. The above equation depends on the

TABLE 4
Fumigant Toxicity of Artificial Limepeel Oil Mixture and Natural Limepeel Oil to *Callosobruchus maculatus* Adults

Treatment	24-h Mortality (95% CL)					
	LC_{50} (μl $litre^{-1}$)	LC_{95} (μl $litre^{-1}$)	Slope ($\pm SE$)[a]	χ^2	DF	TF[b]
Artificial limepeel oil mixture	8·65 (8·35–8·97)	10·64 (10·04–11·77)	18·31(±2·8)	0·70	2	1
Limepeel oil	8·09 (7·78–8·40)	10·27 (9·68–11·30)	15·81(±2·1)	0·23	1	1·07

[a] Data do not contradict the hypothesis of parallelism.
[b] Toxicity Factor (artificial oil as reference).
[c] (+)-limonene (+), γ-terpinene (+), terpinolene (+), α-terpineol (+), *n*-decyl alcohol. (55·0 + 10·6 + 5·3 + 5·8 + 23·3 by mass).

TABLE 5
Relative Fumigant Toxicity of Artificial Limepeel Oil Mixture and its Active Constituents to *Callosobruchus maculatus* Adults

Treatment	24-h Dosage % mortality response (n = 30)		
	8 μl $litre^{-1}$	9 μl $litre^{-1}$	10 μl $litre^{-1}$
Artificial limepeel oil[a]	20	27	97
(+)-limonene + *n*-decyl alcohol (55 + 45 by mass)	0	0	20
γ-terpinene + *n*-decyl alcohol (11 + 89 by mass)	0	0	0
Terpinolene + *n*-decyl alcohol (5 + 95 by mass)	0	0	0
α-terpineol + *n*-decyl alcohol (6 + 94 by mass)	0	0	0

[a] See Table 4

assumption of additive action.[11] The LC_{50} values for all insecticidal components of citrus oil tested, and of all natural oils, were determined by probit analysis (a) above, and proportions of components in citrus oil were determined by GC analysis or from the literature.[2] Data (LC_{50} values and proportions) were then substituted in eqn (1) to estimate the toxicity of the mixture of components not individually examined in this work.

3 RESULTS

3.1 Gas chromatography of citrus oils

It was found that (+)-limonene was the major component of all test oils (lime, lemon, mandarin, orange, grapefruit), although there were other quantitative and qualitative differences observed, consistent with facts published earlier.[2] The percentage compositions of many components tested in this work were extrapolated from GC analysis (see Table 1).

3.2 Fumigant toxicity of citruspeel oil components

Out of 30 components tested, 22 (including (+)-limonene) were shown to have strong fumigant insecticidal activity against *C. maculatus* adults (Tables 1 and 2). The 24-h LC_{50} values of the liquid components ranged from 4·05 (α-terpineol) to 13·13 (*n*-tridecane) μl litre^{-1}; while those of the solids ranged from 4 to 10 mg litre^{-1}. A comparison of the efficacies (based on 24-h LC_{50} values) of limepeel oil (reference citrus oil) and the tested constituents revealed that there were no dramatic differences; for example, the most active component (α-terpineol) was twice as effective as the natural limepeel oil, which was 3·2 times more effective than the least active component (*n*-tridecane) (Table 1). If a component that is 50% of a given mixture is to account for the experimentally observed efficacy of the mixture, then such a component when tested alone would logically be expected to be more effective than the mixture. Applying similar reasoning and computation, it was found (Table 1) that the observed LC_{50} ratios (based on 24-h LC_{50} values of natural oil and of components) were consistently and significantly lower than the expected relative toxicity ratios (based on percentage composition of test components in natural oils) with all bioactive components tested.

3.3 Relative fumigant activity of (+)-limonene (major constituent of all citrus oils)

(+)-Limonene was shown to have a similar toxicity to *S. zeamais* and *D. maculatus* but was significantly more toxic to *C. maculatus* (no overlaps in 95% CL of 24-h LC_{50} values) (Table 3).

3.4 Joint fumigant action of citrus oil components

3.4.1 Accounting for the fumigant activity of a citruspeel oil

Substitution of the LC_{50} values for the components of limepeel oil against *C. maculatus* (Table 1) and their percentage occurrence into eqn (1) (Section 2.6) gives an estimate of the LC_{50} of the untested proportion of the oil as 9·8 μl litre^{-1} (assuming additive action). This untested fraction constituted approximately 25% of the natural limepeel oil.

3.4.2 Artificial limepeel oil mixture

The variance of the LC_{50} values and that of the slope of the probit line for the artificial limepeel oil mixture were not significantly ($P > 0{\cdot}05$) different from those of the natural oil (Table 4); both artificial and natural oils being of the same level of activity. None of the bioactive components included in the artificial oil by themselves or when mixed with the inactive *n*-decyl alcohol, produced a response (mortality) on *C. maculatus* adults proportional to the expected response on a percentage composition basis (Table 5) adopting similar reasoning to that for natural limepeel oil and the 30 components tested (Section 3.2.).

4 DISCUSSION

Out of 30 components tested, 22, including the principal constituent of all citrus oils, (+)-limonene, were shown to have strong fumigant insecticidal activity against *C. maculatus* adults. Joint probit analysis showed that the data for the bioactive liquid components contradicted the model of parallelism. However, for practical purposes, α-terpineol was taken to be the most active liquid component with a toxicity level (LC_{50}) to adult *C. maculatus* twice that of (+)-limonene and 3·2 times greater than that of the least active component, *n*-tridecane (with no overlaps in 95% CL).

Although not directly comparable to this work in which fumigant toxicity was evaluated, earlier work[6] had reported limonene to be the most toxic citrus peel oil component against *C. maculatus* and houseflies when applied by a dry film (filter paper) method in Petri dishes. On the other hand, citral applied by dipping was considerably more toxic than limonene to the Caribbean fruit fly, *A. suspensa*.[7]

All bioactive components (liquids and solids) were found to be volatile at 28°C. This supports the idea that

the insecticide activity of citrus oils depended on their volatile components. However, their bioactivity did not depend merely on the physical property of volatility at NTP. In that case, all volatile compounds would have been expected to be toxic, which was not in the case.

Fumigant activity levels (LC_{50} values or percentage mortality) indicated that none of the individual components tested in this study had a high enough activity against *C. maculatus* to account for the observed effect of limepeel oil on a purely proportional basis (see Table 1 and 2). The LC_{50} values of the various components of citrus oils varied between 4·05 and 13·13 μl litre^{-1}, thereby ranging from below to above the LC_{50} value (7·99 μl litre^{-1}) of limepeel oil (a typical citrus oil). The LC_{50} of the untested proportion, estimated under the supposition of additive action of the components, was slightly higher than that of the oil and there was thus no reason to suppose that this fraction of the oil contained compounds significantly more toxic than those tested. Additive action would seem a reasonable assumption for components with a similar chemical constitution. However, the situation with citrus oils remains unclear, for, while the components of natural citruspeel oils are of varied chemical composition, most are terpenoids. Furthermore, bioassays using artificial and natural limepeel oils gave similar levels of mortality in *C. maculatus* adults. The levels represented in the artificial limepeel oil mixture for each component were below the threshold for toxic action (see regression lines presented for each component tested at a range of higher concentrations, Table 1); and this was the reason for no mortality being recorded for components in Table 5, except for the principal component, (+)-limonene. Thus, it may be inferred that the components are sufficiently similar in action to act accumulatively, i.e. that a total of components individually below threshold combine to give a toxic effect, namely that observed for the mixture. There is still a possibility for some synergistic interaction between small groups of components of citruspeel oils. The fact that the artificial limepeel oil with only four bioactive constituents was as effective as the natural oil is of practical interest. Further work by the author has in fact produced combinations of components of citruspeel oil that significantly synergise their individual fumigant action against insects; there form the subject of a Nigerian patent application.

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